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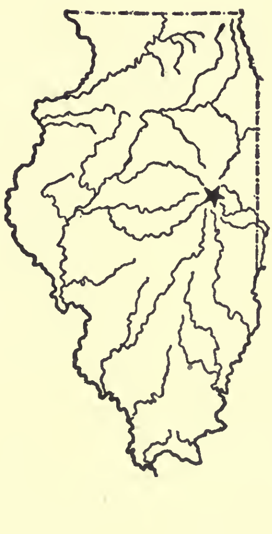
THE INFLUENCE OF PLANT INJURY AND THE ROOT ROT DISEASES UPON THE PHYSICAL AND CHEMICAL COMPOSITION OF CORN GRAIN

By George H. Dungan



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SUMMARY

Constricting the ear-shanks and stalks of corn by breaking the supporting tissue in these structures without completely severing the vascular elements had essentially the same effect upon the yield and composition of corn grain as premature harvesting. Constriction of the shank was much more detrimental to ear development than the breaking of the stalk.

Breaking the shanks when the ears were in the soft-dough stage caused the greatest degree of chaffiness in the grain. Chemical analyses showed that there was no definite correlation between kernel "starchiness" and quantity of starch in the grain, as has been commonly supposed. In fact the quantity of true starch was often less in the "starchy" than in the horny grain. In the light of these results it is suggested that the term "floury" be substituted for the word "starchy" in describing the corn containing more than a normal quantity of soft, dry, friable material.

The percentage of total nitrogen, hemicellulose, and non-hydrolyzable material was distinctly higher in grain from ears produced on broken shanks than in grain from ears produced on sound shanks. The mutilation of the shanks, on the other hand, resulted in a greatly reduced proportion of ether extract and starch in the grain.

Inoculation of the seed at planting time with corn root rot organisms resulted in the production of grain having a specific gravity .027 lower than that from the adjoining uninoculated plants.

The samples of grain produced by plants that grew in the inoculated hills absorbed an average of 5.78 percent more water than the samples from plants growing in uninoculated hills, with odds greater than 9999 to 1 that this difference was not due to chance.

Chemical analyses did not show any significant differences between the grain produced in the inoculated and uninoculated hills. There was slightly more nitrogen, on the average, in the corn from inoculated seed, and a little less ether extract and total sugar. Even tho the average differences in total nitrogen, ether extract, and total sugar were not large, the odds indicating the significance of these results were above 30 to 1.

Analyses of two lots of horny and two lots of floury corn of the Leaming variety showed that there was no significant distinction in chemical composition between these two types of corn. Upon germination, however, the horny corn was found to contain a greater proportion of soluble starch and dextrins than the floury corn, and starch digestion in the horny corn was somewhat more rapid than in the floury corn. This phenomenon offers an explanation for the superior vigor of seedlings from horny corn, so frequently observed.

THE INFLUENCE OF PLANT INJURY AND THE ROOT ROT DISEASES UPON THE PHYSICAL AND CHEMICAL COMPOSITION OF CORN GRAIN

By George H. Dungan, Assistant Chief in Crop Production

INTRODUCTION

For a number of years the Illinois Agricultural Experiment Station has advised farmers to eliminate all extremely "starchy" ears from their seed stock. This recommendation has been based upon the results of investigations into the causes of the corn rot diseases obtained by the Station and the Office of Cereal Investigations of the U. S. Department of Agriculture working cooperatively.

In these investigations by Holbert and associates[13] the fact is established that a high degree of correlation exists between certain ear characters in seed corn and susceptibility of seedlings developed from such ears to infection by root rot organisms. They give the important diagnostic features of diseased seed corn as: (a) a lack of luster in the ear; (b) a discolored or shredded shank attachment; (c) a bleached ear tip; (d) an extremely deep indentation at the crown of the kernel; (e) a relatively high proportion of soft to horny starch in the endosperm; and (f) a dull wrinkled kernel, especially in the region of the germ. It is evident that these characters are not entirely independent of one another, for an increased percentage of soft starch in the endosperm tends to decrease the luster of the ear, and a high content of soft starch is often accompanied also by deep kernel indentation. Thus it would seem that the composition of the grain, especially the proportion of soft to horny starch in the endosperm, is a very important factor, if not the most important single factor, in determining susceptibility or resistance to disease, or at least in indicating such susceptibility or resistance. The data from one experiment in which horny and "starchy" kernels were selected from the same ears and grown in comparative yield trials, showed a reduction of 12 bushels an acre for the "starchy" seed. The results of investigations over a period of seven years showed a decrease of 7.9 bushels an acre for "starchy" seed, with odds greater than one million to one that this difference was not the result of chance.

Trost[38] found that ears of the "starchy" class were characterized by a larger percentage of infection on the germinator than horny

ears from the same seed sample. In experiments using Reid Yellow Dent corn as seed, the "starchy" ears produced a larger number of weak plants and 14 percent lower yield than did ears less "starchy" in composition.

No data, however, have as yet been obtained as to why corn containing a large proportion of soft starch is more susceptible to fungus invasion than corn containing a smaller proportion, and very little direct knowledge is available as to the factors that influence the physical composition of the endosperm in corn grain. In general it may be said that the agencies which determine the type of endosperm in corn divide themselves into two classes; namely, hereditary factors, and those factors that may be included under the general term environment. The present work has been confined to a study of certain factors in the second group; namely, the influence that stalk and shank injury may have at two different stages in the development of the ear, and the effect of the inoculation of the seed at planting time with four organisms, viz., *Diplodia zeae* (Schw.) Lev., *Fusarium moniliforme* Sheldon, *Gibberella saubinetii* (Mont.) Sacc., and *Rhizopus* spp., any one of which is capable of producing a corn root rot disease. Data are also presented on the comparative chemical composition of starchy and horny kernels at different stages during the process of germination.

REVIEW OF LITERATURE

The literature reporting the influence of ecological factors on the physical and chemical composition of cereal grains is voluminous, and this summary is not presented as in any sense exhausting that general subject. Only those papers are reviewed that show the influence of the stage of harvesting and plant injury from mechanical or parasitic causes on the composition of the grain.

The monumental researches of Hornberger[15] in 1882 constitute one of the most intensive pieces of work on the corn plant up to that time. Later Schweitzer,[28] Jones and Huston,[18] and Smith,[32] engaged in the study of the same problem. These workers agreed with Hornberger,[15] who found that as the grain developed beyond the milk stage the proportion of ether extract increased, while the percentage of total nitrogen and ash decreased. They also noted that the relative amount of carbohydrates increased with maturity, while that of the crude fiber decreased. The investigations of Ince[17] also showed a larger proportion of crude fat and nitrogen-free extract in mature grain than in immature grain. Bushey[3] reported that corn cut short in its development by frost contained higher percentages of ash and crube fiber than unfrosted corn. A comparatively high proportion of the nitrogen of the soft corn was in the amid-albumin and globulin forms. Hume, Champlin, and Loomis[16] noted that completely mature corn contained

the largest percentage of oil. Appleman[1] found that the grain of sweet corn decreased markedly in content both of total sugar and of reducing sugar as it developed from the milk to the dough stage. Kent, Patrick, Eaton, and Heileman[20] and Curtiss and Patrick[6] found that after the ears had reached the dough stage there was but little change in the composition of the grain, altho there was a gain in yield per acre.

Analyses reported by Saunders[27] showed that there was a rapid reduction in proportion of crude protein in the wheat grain during its early development, and that afterwards there was a gradual percentage increase in this material. Similar results were also obtained by McDowell[25] in both winter and spring wheat, altho the percentage increase in protein following the early rapid decrease was fluctuating in character.

LeClerc[21] working with wheat, and Failyer and Willard[10] working with both oats and wheat, found that late cutting resulted in a slightly reduced proportion of nitrogen in the grain.

Kedzie[19] made analyses of wheat grain at a great many stages, beginning when the heads were just past the "blossom" stage and continuing until the grain was dead ripe and the straw fallen. His data showed a decrease in the percentage of ash and crude-fiber content as the grain matured. The relative amounts of albuminoids and amid nitrogen decreased rapidly up to the hard-dough stage, after which they increased slightly. The proportion of ether extract decreased until the grain was in the milk stage, after which it rose gradually. The percentage of nitrogen-free extract increased up to the hard-dough stage, then dropped off slightly.

Data presented by Shutt[31] indicated that wheat grain injured by frost before it was completely mature contained a lower percentage of both total nitrogen and albuminoid nitrogen. Blish[2] reported that "frozen wheat contains larger amounts of non-protein nitrogen, reducing sugars, and acid-reacting constituents than does sound wheat. The non-protein nitrogen of the frozen wheat carries a considerably higher percentage of a-amino nitrogen than that of sound wheat."

Severe rust injury hastened the ripening of wheat, and according to Shutt[30] markedly increased the percentage of protein, crude fiber, and ash, and lowered the proportion of fat and nitrogen-free extract. Similar results as to the effect of rust on the ash and protein contents of the wheat grain were obtained by Stoa.[35] Headden,[12] however, stated that the shrunken berries resulting from rust infection were not high in protein. He pointed out that protein and starch were laid down simultaneously, and the effect of rust was to prevent the transfer of the filling material to the berries.

Microchemical studies by Eckerson[9] indicated that starch was formed in the endosperm cells of the developing wheat grain soon after the formation of the cell walls, and that this process continued until

desiccation began. Storage proteins were not formed in the endosperm until the drying of the grain caused the amino acids present to condense into proteins.

MATERIALS AND METHODS

In the experiment to determine the effect of breaking the stalks and ear-shanks, yellow dent corn obtained from James R. Holbert of the U. S. Department of Agriculture, was used as seed. It was of F_1 hybrid material between pure-line strains, and consequently was much more uniform in genetic constitution than average open-pollinated corn. Seed of this corn was planted on the University South Farm and received the usual cultivation under field conditions.

On August 17, when the grain was in the "milk" stage, and also on August 31, after the grain had reached the "soft-dough" stage, a number of the ears were broken down to the extent of markedly constricting the supporting shank but not severing the ear from the stalk. Also, on these two dates, a number of plants were broken over, so that a definite constriction resulted between the nodes of the stalk about one foot from the surface of the ground. The grain from plants so treated was analyzed and the results compared with similar data from ears produced on plants which had not been mutilated.

The seed used in the experiment to determine the effect of plant infection with corn root, stalk, and ear rot-producing organisms on the composition of the grain was obtained from the Plant Breeding Division of the University of Illinois. This seed had been inbred for two generations. Two ears possessed an extremely horny endosperm and two an endosperm containing a high proportion of soft starch. These were planted on May 16, in soil that had grown alfalfa for the twelve years prior to this corn crop. At the time of planting, the alternate hills were inoculated with a pure culture of some one of the following organisms: *Diplodia zeae* (Schw.) Lev., *Fusarium moniliforme* Sheldon, *Gibberella saubinetii* (Mont.) Sacc., and *Rhizopus* spp. The grains were laid, germs up, in the hill. Two horny kernels were placed about an inch apart on the north side of the hill and two "starchy" kernels were similarly placed on the south side of the hill. Two drops of a heavy spore suspension of one of the above-mentioned organisms were placed on the germ face of each kernel in the alternate hills. In order to make sure of getting an infection with *Diplodia zeae* a fragment of shredded cornstalk bearing a pure culture of this organism was placed between the kernels in the hill in addition to the spore suspension placed on the kernels. Two drops of distilled water were placed on the germ face of each kernel in the uninoculated hills. After the moisture in the drops of spore suspension had partially dried down, the corn was covered with moist soil which was pressed firmly down over the seed.

The cultures of *Fusarium moniliforme* and *Rhizopus* spp. were isolated from germinating corn. The cultures of *Diplodia zeae* and *Gibberella saubinetii* (the latter designated as strain 259) were obtained from Miss Helen Johann, of the U. S. Department of Agriculture.

In the study of the progressive chemical changes occurring during germination in horny corn, as compared with those occuring in "starchy" corn, the Leaming variety was used. This lot of corn consisted of twelve ears, six of which were smoothly indented and high in percentage of horny starch and six of which were rough in indentation and relatively high in soft starch. All of these ears had been carefully field-selected early in November and were alike in that they had all been produced on apparently healthy plants.

These ears were divided into four lots of three ears each. Lots A and C were made up of horny ears, and Lots B and D of "starchy" ears (Figs. 1 and 2).

Five sets of germination tests were started in the temperature-control chamber, which was maintained at 16° C. in the plant pathology greenhouse at the University of Wisconsin[a]. Each test contained 20 kernels from each ear, the kernels in each case being removed from the same rows on the ear. The corn was germinated between muslin cloths over moist sand. At the end of the 2d, 4th, 6th, 8th, and 10th days a set of the tests was removed and the kernels or seedlings dried for analysis. Drying was effected by placing the samples in the oven at 100° C. for one hour to kill the enzyms, followed by 18 hours of drying with the oven door open (Link and Tottingham[22]).

CHEMICAL METHODS

All samples of corn grain analyzed were finely ground and passed thru a 100-mesh sieve. The total nitrogen determinations were made by the Official Gunning method modified to include the nitrogen of nitrates.[7] The soluble nitrogen was extracted by soaking the ground grain for four hours in 50 cc. of distilled water to each gram of sample.

The sugars were extracted from the ether-extract-free sample with 90 percent ethyl alcohol, by gently boiling the sample for one hour. After the alcohol was evaporated, the sirupy residue was taken up with water and the solution clarified with neutral lead acetate. The excess lead acetate in the filtrate was then removed by adding sodium sulfate and sodium carbonate in the proportion of nine parts of the former to one of the latter. Phenolphthalein was used to indicate when defecation was complete. After filtering, the reducing sugar was determined, an aliquot of the clear filtrate being used for this purpose. Total sugars were determined after hydrolysis in 2.5-percent hydrochloric acid, by boiling on a sand bath for one hour.

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[a]All the germinative tests and chemical analyses in this investigation were made at the University of Wisconsin.

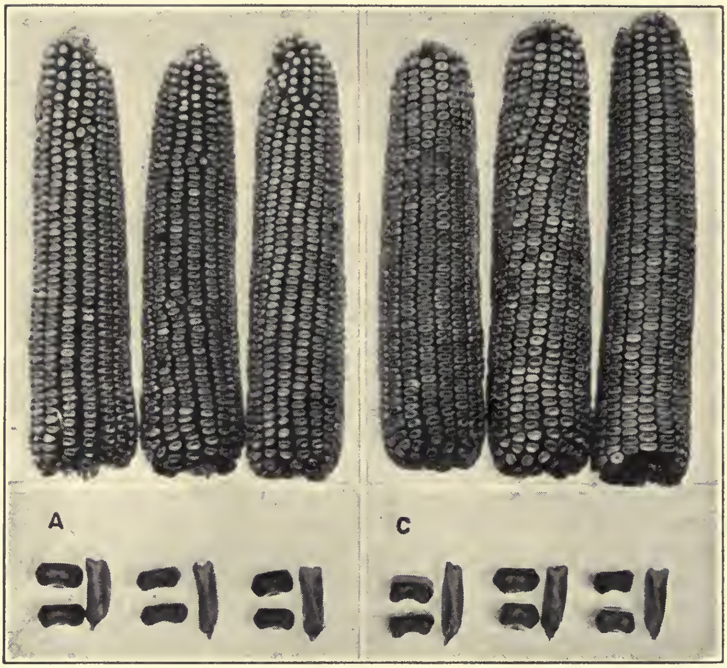

FIG. 1.—HORNY EARS OF LEAMING CORN USED IN STUDY OF CHEMICAL CHANGES OCCURRING DURING GERMINATION

Kernels from these two lots of horny corn and from two lots of "starchy" corn (Fig. 2) were germinated, and the chemical changes taking place during germination were determined by analyzing samples taken at two-day intervals. The results are described on pages 269 to 275.

Dextrins and soluble starch were extracted with cold water in which the sugar-free sample was allowed to soak overnight. The filtrate was hydrolyzed by boiling in 2.5-percent hydrochloric acid for 2½ hours.

The solution containing the insoluble starch was boiled for three minutes to gelatinize the starch present. When cooled to 38° C., fresh saliva was added to hydrolyze the starch to maltose. The complete conversion of starch to maltose was indicated by failure of the material to give a blue color with iodin. The maltose extract was hydrolyzed to glucose by adding hydrochloric acid until the concentration of the acid in the solution was 2.5 percent and by boiling over a sand bath for 2½ hours.

The residue was washed into a flask and submitted to hydrolysis in 2.5-percent hydrochloric acid for one hour. The filtrate was considered to be hemicellulose.

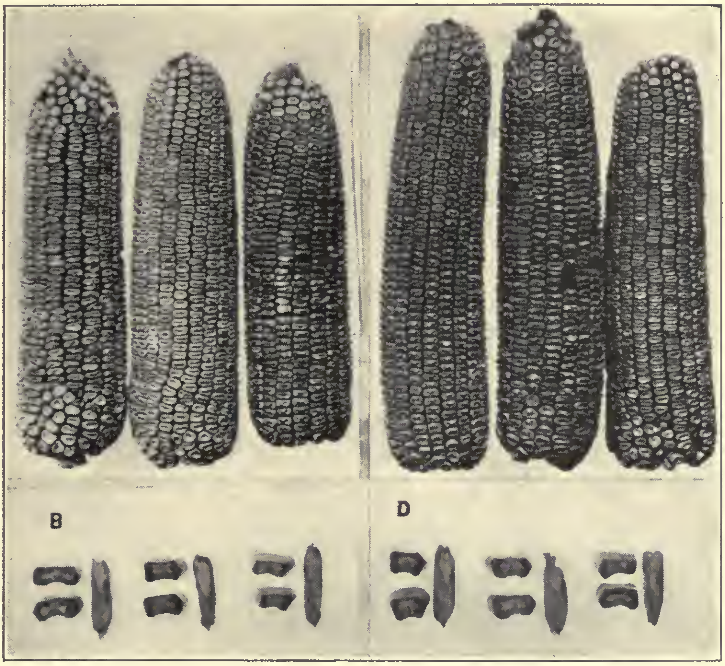

FIG. 2.—"STARCHY" EARS OF LEAMING CORN USED IN THE GERMINATION STUDY

The relative proportion of horny and soft starch in these ears is indicated by the transverse and longitudinal sections of the grain. A preliminary analysis showed practically the same percentage composition for both horny and "starchy" lots, but their specific gravity was markedly different.

The quantity of sugars, soluble starch, and dextrins, insoluble starch, and hemicellulose was ascertained by determining the power of these substances to reduce the copper of Fehling's solution. The amount of copper reduced was measured by the Shaffer-Hartman[29] iodometric titration method.

EXPERIMENTAL RESULTS

Effect of Broken Stalk and Ear Shanks

Weak and diseased corn plants are frequently not strong enough to support the ears to complete maturity; the increasing weight of the ear may exceed the strength of the supporting tissue of the shank, or the stalk itself may be deficient in mechanical substance and break over under stress of wind. To simulate what so often happens in na-

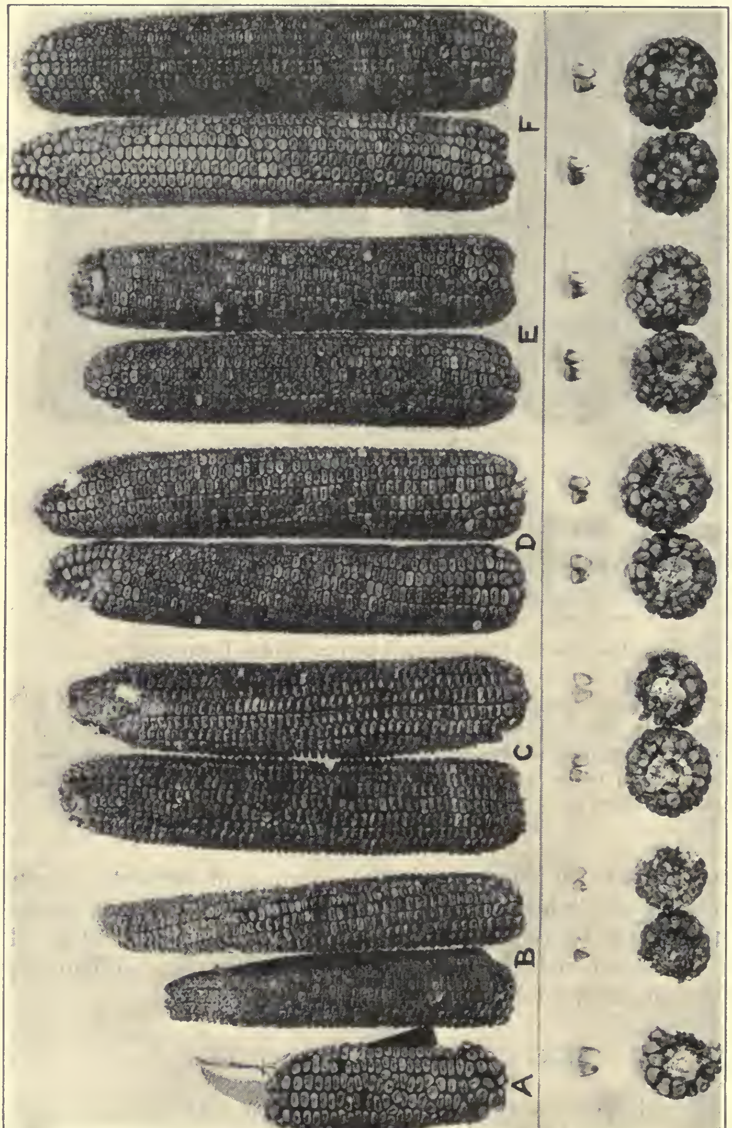

FIG. 3.—REPRESENTATIVE EARS FROM EXPERIMENT ON EFFECT OF BREAKING THE STALK AND EAR SKANK OF CORN PLANTS UPON THE DEVELOPMENT OF EARS AND GRAIN

ture, a number of ear shanks and cornstalks were artificially broken on August 17, when the grain was in the milk stage, and on August 31, when the grain was in the soft-dough stage.

The breaking-over of the stalks and ear shanks did not in any case involve the complete severance of the ear from the shank, nor of the upper portion of the stalk from the stub. The vascular elements, or at least most of them, remained intact. There was, however, a distinct constriction produced, which doubtless interfered greatly with the translocation of reserve and building materials to the ear. It would seem that the constriction of the shank, brought about by breaking down the ears, would be greater than that of the stalk, owing to the fact that the break caused a departure of approximately 120° from the normal position of the shank, whereas the break of the stalk resulted in a declination of only a little over 90°.

That the constriction of the shank and stalk produced a marked effect upon the size and character of the ears and kernels may be observed by reference to Fig. 3 and by a study of the data presented in Table 1.

The breaking of the ear-shank produced a much greater hindrance to the proper filling of the grain than the constriction of the stalk. This is particularly pronounced in the weight of ears, the weight of

TABLE 1.—EFFECT OF BREAKING THE SHANKS AND STALKS OF CORN ON WEIGHT OF EARS (GRAIN AND COB), WEIGHT OF TEN KERNELS FROM EACH EAR, AND GERMINATIVE VIGOR OF THE KERNELS

Group	Treatment	Average weight of ears	Average weight of 10 kernels	Average germination in soil	Average number of secondary roots per seedling	Average length of plumule at end of 8-day germination test in soil
		grams	*grams*	*perct.*		*mm.*
A	Parent ears..............		2.96	98.0	2.88	120
B	Shank broken when grain was in milk stage........	78.4	.69	52.3	1.60	74
C	Shank broken when grain was in soft-dough stage..	191.8	1.59	97.1	1.65	75
D	Stalk broken when grain was in milk stage........	272.4	2.37	88.1	2.67	87
E	Stalk broken when grain was in soft-dough stage..	261.0	2.38	88.3	2.96	100
F	Ears from erect plants and from unbroken shanks...	331.1	2.86	98.1	3.43	117

ten kernels, the number of secondary roots, and the length of plumules. Even tho the delay in breaking the shanks until the soft-dough stage resulted in a much greater weight of ear and kernel, and also increased the percentage of germination, the vigor of the seedlings produced by this corn, as measured by number of secondary roots and length of plumule, was only very slightly better than that of the corn the shanks of which were broken when the grain was in the milk stage.

The chaffy character of the ears in Group C, Fig. 3, is striking. All the ears in this experiment that had their shanks broken when the grain was in the soft-dough stage contained a very high proportion of soft starch. A comparison of the various lots of ears in respect to the relative quantity of soft and horny starch in the endosperm of the kernels is afforded in Table 2.

The parent ears and those that matured on erect plants and on unbroken shanks contained the highest percentage of horny and the

TABLE 2.—EFFECT OF BREAKING THE SHANKS AND STALKS OF CORN AT TWO DIFFERENT STAGES OF GROWTH, ON THE KERNEL CHARACTER OF THE EARS PRODUCED

Group	Treatment	Number of ears represented	Kernel character: percentage of ears that were		
			Horny	Medium	Starchy
A	Parent ears	5	80	0	20
B	Shank broken when grain was in milk stage	13	0	30	70
C	Shank broken when grain was in soft-dough stage	7	0	0	100
D	Stalk broken when grain was in milk stage	10	20	10	70
E	Stalk broken when grain was in soft-dough stage	12	8	0	92
F	Ears from erect plants and from unbroken shanks	11	27	18	55

lowest percentage of "starchy" individuals. However, as may be seen from Table 3, the chemical composition of the kernels in respect to the quantity of true starch which they contained, did not harmonize with their physical appearance.

The early constriction of the shanks resulted in grain high in total nitrogen, hemicellulose, and crude fibrous material that was non-hydrolyzable in 2.5-percent hydrochloric acid and low in ether extract and insoluble starch. When the breaking of the shanks was delayed until the grain was in the soft-dough stage, the proportion of total ni-

trogen, hemicellulose, and non-hydrolyzable material was not so high as when the breaking was done earlier, but it still was considerably higher than in the normally matured ears. There was also a significant increase in the relative amount of ether extract and insoluble starch as the grain developed from the milk to the soft-dough stage.

The breaking of the stalk did not affect the composition of the grain so much as did the constriction of the shank, altho it did result in a slight increase in total nitrogen and non-hydrolyzable material, as well as in a small decrease in ether extract and insoluble starch. The time of breaking the stalks had very little effect on the relative quantity of these materials in the grain. It appears that either the plant above the constriction contained a sufficient amount of previously elaborated food materials to fill out the grain to an almost normal degree, or the bending of the vascular elements in the stalk was not acute enough to prevent the passage of solutions for synthetic pur-

TABLE 3.—EFFECT OF THE BREAKING OF THE SHANKS AND STALKS OF CORN ON THE CHEMICAL COMPOSITION OF THE CORN GRAIN
(Amounts expressed in percentages)

Group	Treatment	Total nitrogen	Ether extract	Total sugar	Dextrins and soluble starch	Insoluble starch	Hemicellulose	Material non-hydrolyzable in 2.5-percent HCl
A	Parent ears..................	2.07	4.83	1.32	2.01	53.17	6.62	6.16
B	Shank broken when grain was in milk stage..............	3.40	3.16	1.17	1.76	39.76	9.55	9.90
C	Shank broken when grain was in soft-dough stage..........	2.27	3.92	1.54	1.44	44.71	9.04	7.68
D	Stalk broken when grain was in milk stage................	1.90	4.18	1.05	1.78	51.85	6.65	6.19
E	Stalk broken when grain was in soft-dough stage............	1.85	4.38	1.53	1.77	51.61	7.25	6.81
F	Ears from erect plants and from unbroken shanks............	1.66	4.56	1.61	2.67	54.35	6.66	5.95

poses into the lodged plant. It is very likely that both the translocation of storage material already in the stalk and the deposition of reserves synthesized after the stalk was broken were contributing factors in producing grain of a practically normal composition. Even tho the relative amounts of materials in the grain produced on broken stalks closely approached that of the checks, the total weights of ears and grains were considerably below those of the checks (Table 1).

A comparison of the data presented in Table 2 with that in Table 3 brings out the fact that the condition of the corn grain which has so often been described by the term "starchy" is not necessarily associated with a higher percentage of starch in the grain, but that, on the

contrary, there is a slight suggestion that the ears which are more "starchy" in appearance may actually contain less starch hydrolyzable with saliva than horny ears of the same variety.

EFFECT OF PLANT INFECTION WITH CORN ROOT ROT ORGANISMS

Not all the plants that were inoculated with the four corn root parasites gave evidence of being injured appreciably by these organisms. In order to obtain data on the effectiveness of the inoculation, both shelled grain weights and root anchorage figures were taken. The

TABLE 4.—SPECIFIC GRAVITY OF THE GRAIN PRODUCED FROM INOCULATED HILLS COMPARED WITH THAT OF GRAIN FROM PLANTS IN ADJACENT, UNINOCULATED HILLS

Organism with which seed was inoculated	Number by which inoculated plant was designated	Specific gravity of grain from— Inoculated hill	Specific gravity of grain from— Adjoining uninoculated hill	Difference in favor of uninoculated hill
Diplodia zeae	2b	1.111	1.116	.005
	9b	1.096	1.042	−.054
	27b	1.085	1.126	.041
	37b	1.132	1.161	.029
	55b	1.053	1.188	.135
	Average	1.095	1.127	.032
Fusarium moniliforme	3a	1.222	1.302	.080
	10a	1.217	1.243	.026
	28a	1.214	1.245	.031
	56a	1.312	1.302	−.010
	3b	1.090	1.100	.010
	10b	1.127	1.117	−.010
	17b	1.066	1.040	−.026
	28b	1.167	1.196	.029
	38b	1.193	1.298	.105
	Average	1.178	1.205	.027
Gibberella saubinetii	54a	1.281	1.293	.012
	12b	1.047	1.069	.022
	19b	1.142	1.132	−.010
	40b	1.188	1.239	.051
	54b	1.233	1.320	.087
	Average	1.178	1.211	.033
Rhizopus spp	18a	1.243	1.243	.000
	25a	1.173	1.226	.053
	4b	1.063	1.066	.003
	25b	1.040	1.040	.000
	32b	1.219	1.239	.020
	Average	1.148	1.163	.015
Grand average		1.154	1.181	.027

Odds that the average difference is not due to chance = 302:1

root anchorage was determined by measuring the pulling resistance of the hills at harvest time. Grain from only those plants that yielded less, or that had a root anchorage below that of the adjoining uninoculated check, was selected for analysis. The yield, however, was given primary consideration in making this selection. No ear was taken for this study unless its weight was less than its check.

It was possible in many instances by visual comparison to detect a slightly greater proportion of soft starch in the corn from the inoculated hills. This was not possible, however, in every case. In order to determine more accurately the influence that infection had upon the density of the grain, specific gravity determinations by displacement in 95-percent alcohol were made. The results of this experiment are given in Table 4.

In only five cases out of the twenty-four sets tested did the grain

TABLE 5.—PERCENTAGE OF WATER ABSORBED BY GRAIN PRODUCED ON CORN PLANTS GROWN FROM INOCULATED SEED, COMPARED WITH THAT ABSORBED BY GRAIN PRODUCED ON PLANTS FROM UNINOCULATED SEED

Organism with which seed was inoculated	Number by which inoculated plant was designated	Amount of water absorbed in 24 hours by grain produced on plants from—		Difference in favor of corn from inoculated seed
		Inoculated seed	Adjoining uninoculated seed	
		perct.	*perct.*	*perct.*
Diplodia zeae	27b	72.53	64.16	8.37
	37b	64.27	63.09	1.18
	55b	67.82	62.28	5.54
	Average	68.21	63.18	5.03
Fusarium moniliforme	3a	53.09	49.26	3.83
	28a	51.40	44.89	6.51
	56a	55.41	49.42	5.99
	3b	65.26	58.97	6.29
	10b	61.32	62.57	−1.25
	28b	73.06	54.53	18.53
	38b	54.87	47.86	7.01
	Average	59.20	52.50	6.70
Gibberella saubinetii	54a	47.58	48.34	− .76
	12b	72.03	63.12	8.91
	19b	67.57	64.97	2.60
	40b	62.91	54.66	8.25
	54b	56.62	48.59	8.03
	Average	61.34	55.93	5.41
Rhizopus spp	4b	72.04	65.04	7.00
	32b	56.99	54.66	2.33
	Average	64.52	59.85	4.67
Grand average		62.04	56.26	5.78

Odds that this difference is due to inoculation > 9999:1

from the inoculated plant exceed that of the check in specific gravity. This occurred once with Diplodia, three times with Fusarium, and once with Gibberella. In every case, however, the average specific gravity was higher for the corn from uninoculated plants, and when the results from all the organisms were averaged the grain from uninoculated plants was found to be .207 higher than the specific gravity of the infected plants, with odds of 302 to 1 that this difference was not due to chance.

In some previous work by the author[8] it was shown that corn possessing a high proportion of soft starch in the endosperm is capable of

TABLE 6.—RESULTS OF CHEMICAL ANALYSES OF CORN GRAIN PRODUCED BY PLANTS GROWN FROM INOCULATED AND FROM UNINOCULATED SEED
(Results expressed in percentages)

Kind of corn	Plant No.	Total nitrogen	Ether extract	Total sugar	Dextrins and soluble starch	Insoluble starch	Hemicellulose	Non-hydrolyzable residue
Diplodia-inoculated....	2b....	2.77	4.26	1.32	2.58	48.22	7.72	5.94
Adjacent check......	6b....	2.22	4.70	1.68	3.15	50.06	7.91	5.32
Diplodia-inoculated....	9b....	2.26	4.67	1.13	1.89	51.23	7.67	5.87
Adjacent check......	13b....	1.81	4.61	1.39	1.75	52.92	7.36	6.49
Fusarium-inoculated...	10a....	2.31	4.07	1.23	2.48	48.93	6.13	4.52
Adjacent check......	14a....	1.99	4.42	1.53	2.67	51.30	6.46	4.35
Fusarium-inoculated...	17b....	1.64	4.45	1.32	1.73	53.32	8.29	6.33
Adjacent check......	21b....	1.84	4.74	1.34	1.82	53.21	7.53	5.95
Gibberella-inoculated..	1a....	1.55	3.73	.95	3.07	61.96	5.85	5.33
Adjacent check......	5a....	1.68	4.12	.95	2.43	55.76	5.91	5.56
Gibberella-inoculated..	1b....	2.92	4.44	1.03	2.71	45.65	8.16	7.76
Adjacent check......	5b....	1.79	5.16	1.24	2.76	56.97	7.45	6.25
Rhizopus-inoculated...	18a....	2.02	5.01	1.70	3.92	52.94	5.74	4.81
Adjacent check......	14a....	1.99	4.42	1.53	2.67	51.30	6.46	4.35
Rhizopus-inoculated...	25a....	2.37	4.02	1.08	2.30	52.58	6.57	7.29
Adjacent check......	21a....	2.09	4.38	1.13	2.43	55.28	6.79	4.70
Rhizopus-inoculated...	25b....	1.87	4.65	1.29	2.01	53.92	6.94	5.14
Adjacent check......	21b....	1.84	4.74	1.34	1.82	53.21	7.53	5.95
Average difference in favor of inoculated plant...............		+ .31	− .22	− .12	+ .13	−1.14	− .14	+ .45
Odds that above difference is not due to chance............		44:1	57:1	57:1	4:1	4:1	5:1	18:1

more rapid water absorption than corn containing a less amount of this material. Accordingly, the water-absorptive capacity of the corn in the present experiment was determined with a view to applying the results as a measure of soft-starch content. The data included in Table 5 were obtained by soaking samples of corn produced by inoculated and check plants, in distilled water, under the same conditions, for a period of twenty-four hours. The percentages are based on the water-free weight of the sample.

In every instance except two—one with Fusarium and one with Gibberella—the corn from infected plants absorbed more water than that from the adjoining checks. When all the determinations with corn from plants inoculated with the four organisms were averaged, the difference between the percentage of water absorbed by the grain from inoculated plants and that by grain from the checks was 5.78, and the odds were greater than 9999 to 1 against a difference as great as this being due merely to chance.

Notwithstanding this conclusive evidence, the results of the chemical analyses of representative samples, as presented in Table 6, do not show any marked differences between corn grain produced by plants from inoculated seed and those from uninoculated seed.

When the differences between the two types of grain in all the nine pairs of determinations are averaged, it is found that the corn from the inoculated plants contained .31 percent more nitrogen, .13 percent more soluble starch and dextrins, and .45 percent more non-hydrolyzable substance than the accompanying checks. The odds that these average differences were not due to chance were 44 to 1, 4 to 1, and 18 to 1, respectively. The corn from the uninoculated plants exceeded that from the inoculated plants in ether extract by .22 percent, with odds of 57 to 1. The average difference in total sugar was .12 percent in favor of the uninoculated checks, and this result also carried odds of 57 to 1. Insoluble starch and hemicellulose averaged slightly higher for the check corn, but the significance of these results as measured by odds is very low.

CHEMICAL CHANGES OCCURRING IN "STARCHY" AND HORNY CORN DURING THE GERMINATIVE PROCESS

A preliminary analysis of the four lots of Leaming corn represented in Figs. 1 and 2 showed that the "starchy" lots, B and D, had practically the same percentage composition as the horny lots, A and C. That there was a marked difference in these lots of corn may be seen from their specific gravity, a record of which is given in Table 7.

TABLE 7.—SPECIFIC GRAVITY OF THE GRAIN OF LEAMING CORN, TWO LOTS OF WHICH WERE HORNY AND TWO "STARCHY"

Type of corn	Lot	Weight of 20 kernels	Specific gravity	Average specific gravity of the two lots of the same type
		(grams)		
Horny..................	A..........	7.85	1.312	1.292
	C..........	7.87	1.271	
"Starchy"..............	B..........	6.54	1.208	1.204
	D..........	6.40	1.199	

In order to obtain data on the difference in quality of the materials contained in these two types of seed, a germination test of both was made at 16° C., and the kernels and the seedlings with grains attached were sampled for analysis every two days. The degree of development is illustrated in Fig. 4. The results obtained are presented in Table 8. The data from Lots A and C and from Lots B and D are averaged and set forth in this table under the descriptive heads, horny and "starchy," respectively.

TABLE 8.—CHEMICAL COMPOSITION OF THE CORN, GRAIN, AND SEEDLINGS OF HORNY AND "STARCHY" CORN DURING THE PROCESS OF GERMINATION AT A CONSTANT TEMPERATURE OF 16° C.

Material	Type of corn	Percentage composition of seedlings after being on the germinator for different numbers of days					
		0 days	2 days	4 days	6 days	8 days	10 days
Ether extract.....	Horny....	4.81	4.81	4.74	4.58	4.21	4.03
	"Starchy".	4.96	4.95	4.87	4.64	4.31	4.00
Total nitrogen....	Horny....	1.94	1.94	1.94	1.93	1.90	1.91
	"Starchy".	1.89	1.85	1.88	1.85	1.79	1.85
Soluble nitrogen...	Horny....	.24	.19	.21	.33	.41	.53
	"Starchy".	.25	.20	.24	.35	.42	.53
Total sugar.......	Horny....	1.27	1.34	1.66	2.91	3.87	5.66
	"Starchy".	1.26	1.26	1.27	3.00	3.42	6.10
Reducing sugar...	Horny....	.15	.68	1.47	1.84	2.53	3.71
	"Starchy".	.02	.57	1.04	1.92	2.40	3.98
Dextrins and soluble starch......	Horny....	2.73	2.67	2.92	3.22	3.76	3.20
	"Starchy".	2.90	1.92	2.52	2.41	2.64	2.45
Insoluble starch...	Horny....	51.52	50.16	50.54	49.49	47.23	45.57
	"Starchy".	50.91	50.45	50.82	50.06	47.78	46.48
Hemicellulose.....	Horny....	6.96	7.12	7.13	7.10	7.26	7.35
	"Starchy".	7.15	7.34	7.51	7.48	7.34	7.82

From the second day to the end of the period there was a gradual but distinct reduction in the percentage of ether extract in both the horny and the "starchy" groups. This decrease is illustrated graphically in Fig. 5. These data harmonize with the results obtained by Toole,[37] who found that the fat in the maize embryo was rapidly oxidized during the germinative process.

The fact that the "starchy" corn possessed a slightly greater percentage of ether extract than the horny ears should not be taken as of any particular significance, for the "starchy" Lot B contained somewhat less fat than either Lots A or C. Lot D, however, was abnormally high in fat, and this contributed to the slightly superior average

of the "starchy" group over the horny group. It is also probable that the more rapid exhaustion of the fat of the "starchy" kernels is of doubtful import, since the differences between these percentages are well within the error common to these determinations.

There was a very slight, tho fluctuating, reduction of total nitrogen during germination. The proportion of soluble nitrogen, however,

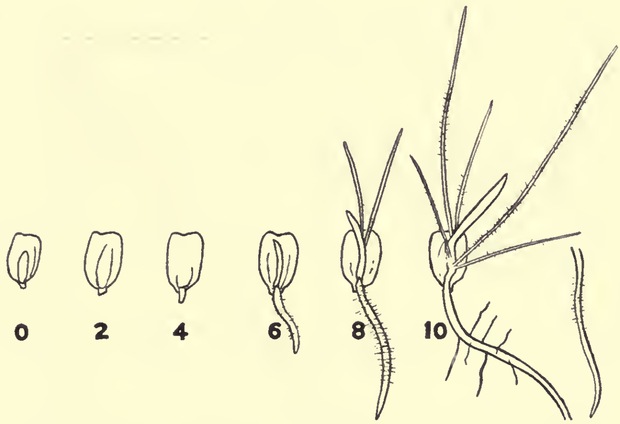

FIG. 4.—APPROXIMATE AVERAGE GROWTH OF THE SEEDLINGS TAKEN FROM THE GERMINATOR AT TWO-DAY INTERVALS

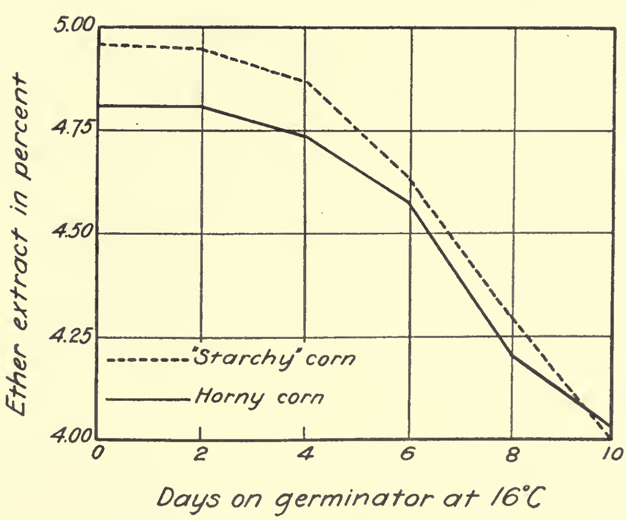

FIG. 5.—PERCENTAGES OF ETHER EXTRACT IN HORNY AND IN "STARCHY" CORN AT DIFFERENT STAGES OF GERMINATION

after a small decrease at the end of the second day, increased appreciably thereafter. It is worthy of note that the soluble nitrogen was consistently higher in the "starchy" than in the horny corn. When the soluble nitrogen was calculated as a percentage of the total nitrogen, the greater solubility of the nitrogen of the "starchy" type of corn is more strikingly emphasized. Fig. 6 was prepared from such a calculation.

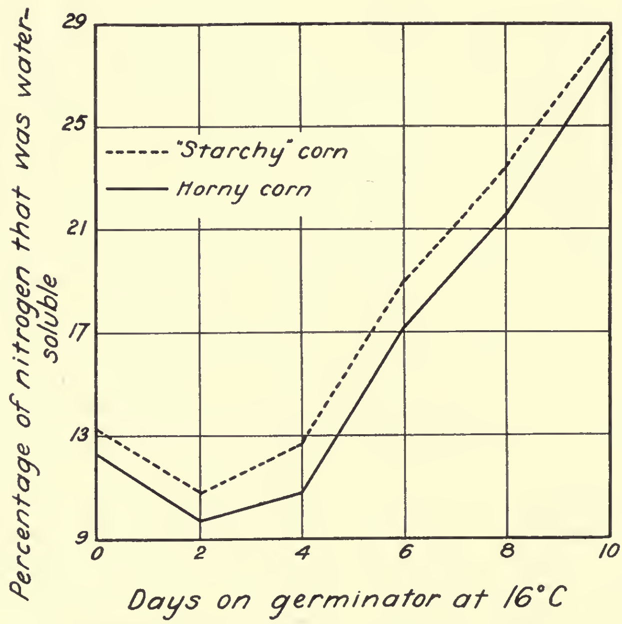

FIG. 6.—PROPORTIONS OF TOTAL NITROGEN THAT WERE WATER-SOLUBLE IN HORNY AND IN "STARCHY" CORN AT DIFFERENT STAGES OF GERMINATION

A larger quantity of the nitrogen contained in the "starchy" corn was in a soluble form than that in the horny corn. In this respect the "starchy" corn resembles grain lacking in maturity. There is the suggestion that the nitrogen of the "starchy" corn is more readily converted to the soluble form during the process of germination than is the case with horny corn.

The amount of total sugar in the horny corn increased from the very beginning of germination. The rise was more gradual, however, during the early than during the later stages of germination. The increase of sugar in the "starchy" corn was initiated somewhat more slowly than that in the horny corn, altho at the end of the sixth day the amount of total sugar in the "starchy" corn slightly exceeded that

in the horny corn. A comparison of these two types of corn in respect to total sugar content may be made from Fig. 7.

The increase in reducing sugar was much more marked from the very beginning of germination than was the case with total sugar. At the end of the second day, when the total sugar was increased only .07

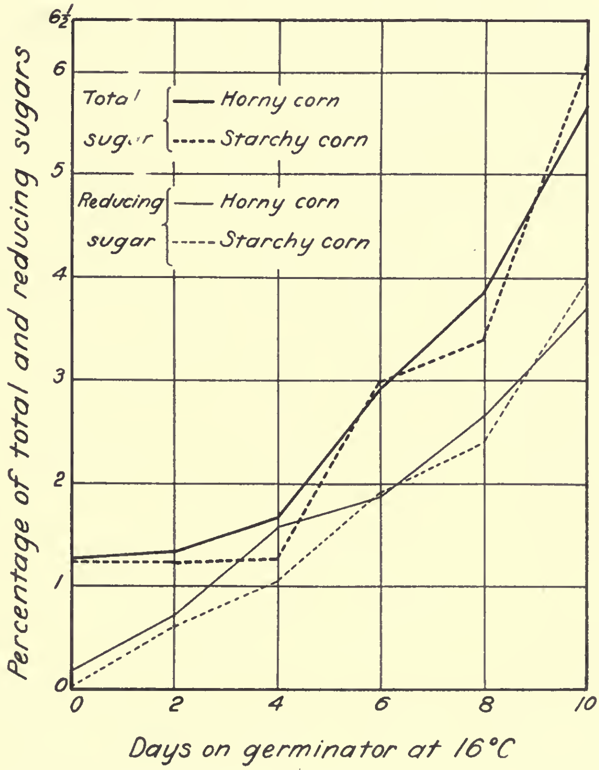

FIG. 7.—PERCENTAGES OF TOTAL AND REDUCING SUGARS IN HORNY AND IN "STARCHY" CORN AT DIFFERENT STAGES OF GERMINATION

There was no uniform difference in the sugar content of the horny and starchy corn during the process of germination. On the 6th and 10th days of germination the percentages both of total and of reducing sugars were higher in the "starchy" corn than in the horny corn. The reverse was true at the beginning of the test and at the end of the 2d, 4th, and 8th days of germination.

of one percent in the horny corn, and not at all in the "starchy" corn, the amount of reducing sugar was multiplied 4½ times in the horny

corn and 28 times in the "starchy" corn. Fig. 7 shows that the relation of the quantity of reducing sugar in the two types of corn was roughly the same as that of the total sugar. It is evident from this chart that the proportion of reducing sugar to total sugar increased markedly until the fourth day of germination, after which time there was a reduction in this proportion. The ratio of reducing sugar to total sugar approached the 65-percent mark on the sixth day of germination, and remained there with minor variations to the end of the period.

It is significant that the soluble starch and dextrins were consistently higher in the horny group than in the "starchy" lot. The quantity of these materials was somewhat greater in the "starchy" lots,

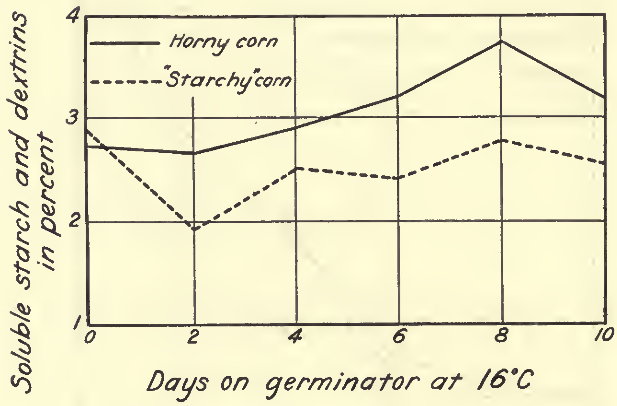

FIG. 8.—SOLUBLE STARCH AND DEXTRINS IN HORNY AND IN "STARCHY" CORN AT DIFFERENT STAGES OF GERMINATION

Soluble starch and dextrins are intermediate products in the conversion of starch to sugar. Their accumulation in greater quantities in the horny corn during germination is suggestive either of a greater ease of starch hydrolysis or of a less rapid utilization of the final products of hydrolysis by the growing seedling. The evidence here presented indicates that the former is nearer the truth than the latter.

however, at the beginning of the germination test. The uniform continuity of this greater proportion of soluble starch and dextrins in the horny group is shown in Fig. 8.

The reduction in percentage of insoluble starch is represented in Fig. 9. From this it is evident that the digestion of starch reserves in the endosperm of the horny grain progressed at a slightly more rapid rate than in the "starchy" grain. This difference in unhydrolyzed starch in the two types of corn is in accord with the data already pre-

sented, showing the greater proportion of soluble starch and dextrins in the horny than in the "starchy" corn. Holbert and associates[13] and Trost[38] have reported that the seedlings and plants produced from

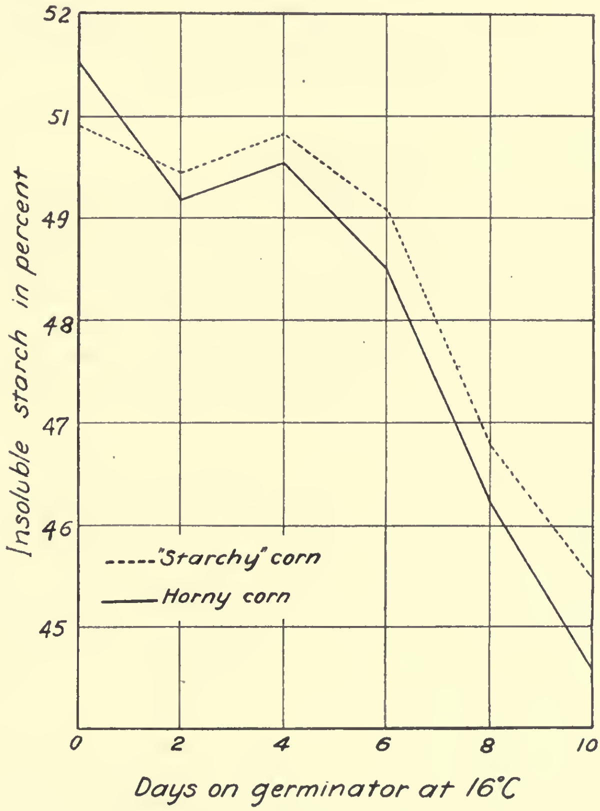

FIG. 9.—PERCENTAGES OF INSOLUBLE STARCH IN HORNY AND IN "STARCHY" CORN AT DIFFERENT STAGES OF GERMINATION

Striking as it may seem, the horny corn contained slightly more starch at the beginning of the test than the "starchy" corn. From the 2d until the 10th day of germination, however, the "starchy" corn contained a greater percentage of starch than the horny. This suggests that the starch of the horny corn is more readily hydrolyzed to soluble forms than that of the "starchy" corn.

seed corn having a horny composition were superior in vigor and possessed a greater resistance to certain corn diseases than seed corn having a "starchy" composition. The more ready solubility of the starch in

the horny endosperm corn than in the "starchy" corn is believed to be an important contributing factor to this difference in vigor and disease resistance.

The data on the quantity of hemicellulose fail to show any uniform change in this material during germination, altho on the whole it did increase proportionately, owing in all probability to the oxidative consumption of other carbohydrate materials.

DISCUSSION

A comparison of the composition of the grain from the broken stalks and ear shanks in the present experiment with the results obtained by Hornberger and Raumer,[15] Schweitzer,[28] Jones and Huston,[18] and Smith[32] shows that this treatment had essentially the same effect upon the grain as premature harvesting. There was an increase in starch content as a result of allowing the ears to remain on unbroken shanks from the milk stage to the soft-dough stage, and a still greater starch deposition when the ears were permitted to go to maturity without shank mutilation (Table 3).

It is striking, however, that in those samples broken at the intermediate stage, the physical appearance of the corn was such as to be classed as 100-percent "starchy," whereas those ears which contained more real starch were apparently not so "starchy" (Table 2). Furthermore from the results presented in Table 8 it is plain that corn with a high proportion of soft starch in the endosperm is not necessarily high in actual content of starch. There is evidently a physical difference in these two types of corn which represents a qualitative dissimilarity as well as a quantitative chemical one.

These results suggest that the "starchy" condition may be induced or accentuated by many ecological factors. Interference with the usual translocative processes, brought about by breaking the stalks, increased materially the proportion of soft starch in the endosperm. Premature harvesting doubtless produces the same effect. The injury to the shanks which greatly increased "starchiness," produced an effect not unlike that of complete removal of the ear from the plant. The modification of the plant's metabolism caused by infection with the corn root rot organisms also increased the "starchiness" of the grain, and the root rot diseases are associated with both hastened and delayed maturity of the grain. It would seem that even slight alterations from the optimum environment, either of parasitic or climatic factors and possibly edaphic factors, may have a distinct effect upon the quality of the grain.

Snyder[33, 34] explains that the light weight of the apparently "starchy" wheat grains is due to a lower than average degree of matur-

ity, as evidenced by their high nitrogen, phosphoric acid, and potassium content.

Roberts and Freeman [26] draw the conclusion that it is the presence of air-vacuoles that gives to yellow-berry wheat its "starchy" appearance and its uniformly low specific gravity. They support their reasoning by stating that the specific gravity of starch is 1.53, sugar 1.60, and cellulose 1.53, whereas gluten, which is greater in amount in the horny grain, has a specific gravity of only 1.297. An increased amount of a substance having a lower specific gravity would not of itself increase the specific gravity of the whole. Hence, it was assumed that the lesser specific gravity was due to air-vacuoles.

Cobb[4, 5] and Lyon and Keyser[23] point out that the starch granules in soft wheats are larger in diameter than those of horny wheats. Even tho the horny wheat varieties contain some large-sized starch granules, their relative number is much less than in the softer varieties. Cobb[5] also notes that "whenever the starch grains are large, the cells containing them are also large." Lyon and Keyser[23] working with yellow-berry in wheat, state that "the protoplasmic network of the cells in the sections from the very horny kernels showed only an occasional vacuole. Sections from the markedly yellow kernels showed very much more numerous and larger vacuoles."

Hackel[11] attributes the filling up of the intervals between starch grains to albuminoids. The analyses of Hopkins, Smith, and East[14] support this view by showing that the horny starch of the corn grain contains 2.3 percent more protein than the white starch of the same grain. Their data also show that the horny starch contains slightly more oil.

An investigation into the nature of the starch in horny and in "starchy" corn would seem to offer a very promising approach to the explanation of the real difference between these distinct types of dent corn. Tanret,[36] from a study of starches in oats, bananas, wheat, chestnuts, beans, lentils, maize, barley, peas, apples, rice, buckwheat, rye, and potatoes, concluded that the starches from these various sources are not only dissimilar chemically, but that they react differently to physical and other agents. Since the various grains and fruits contain different kinds of starch, it is probable that there may be many different kinds of starch within the same grain, and when the proportions of these vary the character of the grain is changed.

That there is a difference either in the ease with which starch is hydrolized or in the activity of the dissolving enzym is indicated by the consistently superior quantity of soluble starch and dextrins in the horny corn over the "starchy" corn during germination. This property is in all probability responsible for the greater vigor of corn seedlings from the horny seed.[13]

The immediate problem confronting the research worker in this field appears to be not so much the question of what factors tend to favor the development of the "starchy" character of corn grain, but what "starchiness" really is as contrasted with horniness, the influence which this condition has upon the metabolic processes incident to seedling production, and the ease or difficulty with which seedlings are infected by fungi.

In view of the fact that the difference between horny and "starchy" is not one of quantity of starch, it would seem desirable to discontinue the use of the term "starchy" as descriptive of that type of corn and to substitute an adjective which is accurately descriptive of the condition. A number of words suggest themselves, but cannot be used because they have previously been adopted to describe other conditions. While "soft" is often used to carry a meaning the opposite of horny, common usage has led to the very general application of the term to immature corn having an unusually high percentage of moisture. The adjective "mealy" would be a good term to substitute for "starchy," but it has been used by Mangelsdorf[24] to designate a particular type of endosperm in which practically all the material of which it is composed is soft starch. The term "floury" is therefore suggested by the author as the most nearly appropriate word for describing corn grain which has a relatively large quantity of soft starch in the endosperm. "Floury" connotes the qualities of flour; namely, light or pale-colored, soft, and powdery. "Floury" corn, then, would be maize grain that is made up largely of material having a pale color and a soft and friable texture. The distinction between "floury" corn, as it is understood here, and the flour, or soft, corn of the tropics should be kept in mind. "Floury" corn is corn of the dent type possessing a relatively high proportion of soft starch in the endosperm, whereas flour corn is a separate and distinct type of corn, the grains of which are very large and the endosperms of which contain no horny material.

CONCLUSIONS

The results of these investigations indicate that the character of the starch in the endosperm of corn grain may be influenced to a perceptible degree by the environment in which it is produced. The inoculation of the seed with certain organisms capable of causing the corn root rot disease alters the physiology of the plant produced by such seed, resulting in the development of grain containing a greater percentage of floury starch than that produced by plants from uninoculated seed. Injury of the plant, such as the breaking of the shanks and stalks, before the grain is completely mature causes an

increased proportion of white starch in the grain of such plants. If, however, the shank be broken when the ear is in a very immature condition, a grain possessing a waxy rather than a floury endosperm may result.

Seedlings produced by corn possessing a relatively large quantity of soft starch in the endosperm are not so vigorous as are those from horny corn, owing to the fact that the floury starch is less rapidly hydrolyzed to a soluble condition than the horny starch.

In general, horny corn contains as much starch as, and frequently more than, floury corn of the same variety, but the specific gravity of floury corn is much lower than that of horny corn.

A quantitative determination of the water absorbed by different lots of corn under the same conditions furnishes a good index as to the comparative amounts of soft starch contained in the samples.

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